REMARKS

This RCE is filed in response to the Decision of the Board of Patent Appeals and Interferences ("the Board") dated June 11, 2008. Applicant submits herewith an appropriate amendment to the claims so rejected and arguments relating to rejections affirmed or newly raised by the Board.

Amendments to Specification

The specification has been amended to correct a sequence discrepancy in SEQ ID NO:1 by inserting a nucleotide "T" at the end thereof. The discrepancy is that SEQ ID NO:1 is described as being a 13mer in paragraphs [0025], [0054] and [0058] of the instant specification; whereas the actual sequence listed in paragraphs [0025] and [0054] as well as in the Sequence Listing has only 12 nucleotides.

Applicant respectfully submits that the nucleotide "T" was inadvertently left out from the end of SEQ ID NO:1 and that such omission is merely a typographic error. For support, Applicant directs the Examiner's attention to the article of Sando and Kool (*J. Am. Chem. Soc.* 124(10): 2096-2097, 2002; previously cited by the Examiner in the Office Actions dated April 18, 2006 and October 17, 2006) in addition to the above-mentioned paragraphs of the instant specification. The article of Sando and Kool (a copy enclosed herewith for the Examiner's convenience) was cited in paragraph [0018] of the instant specification and published on Web on February 13, 2002. One of ordinary skill in the art would readily know, at the time of the filing of the present invention, that SEQ ID NO:1, which represents the sequence of an electrophilic 13mer probe, would have the sequence of 5'-TGT*GGGCAAGAGT-3' as clearly illustrated in the above article of Sando and Kool, in particular, Figure 1(b). In fact, in the Rule 132 Declaration that was submitted along with a

response filed by Applicant on January 10, 2007, Dr. Eric T. Kool (the sole inventor of the present invention) stated that the above article of Sando and Kool described his own work on which the instant application was based. A copy of that Declaration is again enclosed herewith for the Examiner's convenience.

In addition, the Sequence Listing on file has been replaced with the substitute Sequence Listing enclosed herewith, which corrects the above-mentioned error in SEQ ID NO:1. The specification has further been amended by directing the entry of the substitute Sequence Listing therein.

Further submitted herewith are both computer readable form (CRF) and paper form of the substitute Sequence Listing, which is also updated using PatentIn Version 3.5. The statements required under 37 CFR §1.821(f) and (g) and §1.825(d) are submitted concurrently.

Applicant respectfully submits that the foregoing amendments to the specification including Sequence Listing do not introduce any new matter to the application as originally filed.

Claim Status

Claim 1 has been amended to incorporate the subject matter of claim 15, which claim has subsequently been cancelled.

Claim 23 has been amended to correct an apparent clerical error.

Claim 28 has been cancelled as being redundant from claim 26.

Claims 17, 21 and 25 have been amended to refer to the composition of claim 1.

New claim 50 has been added to further characterize that the fluorescence quenching leaving group is a dabsyl group. Support for such claim is found throughout the instant specification and in original claim 15.

Applicant respectfully submits that the foregoing amendments do not introduce any new matter to the application as originally filed. It is noted that new claim 50 falls within the scope of the elected invention, which is directed to the product. With the foregoing amendments, claims 1-14 and 16-27 and 29-50 are currently pending, among which claims 16-27 and 29-39 have previously been withdrawn by the Examiner as being drawn to non-elected species and inventions as indicated in the second Advisory Action dated March 13, 2007.

Applicant notes that claim 15 was previously objected to by the Examiner as indicated in the second Advisory Action dated March 13, 2007. The undersigned called the Examiner inquiring the reasoning for the objections to claim 15 on March 15, 2007. In her telephone message left with the undersigned later that day, the Examiner stated that claim 15 was not rejected as it was not involved in any of the remaining rejections, but it was objected to since it depended on rejected claim 1. It appears that the Examiner would consider claim 15 allowable if rewritten to incorporate limitations of the base claim. Applicant respectfully submits that claim 1 has been amended herein to incorporate the subject matter of claim 15 and as such, should be allowable.

Applicant also notes that claims 40-49 were previously allowed in the second Advisory Action dated March 13, 2007.

Affirmed Claim Rejection – 35 USC §102

Claims 1-8, 10, 11 and 14 are rejected under 35 USC §102(a) as allegedly being anticipated by Livak et al. (PCR Meth. Appl., vol. 14, pp. 357-362, 1995, "**Livak**"). Applicant respectfully traverses this rejection.

Applicant first notes that claim 1 has been amended to incorporate the subject matter of claim 15, which subject matter was previously indicated allowable by the Examiner. As amended, claim 1 is directed to a composition comprising a fluorophore compound, which comprises a fluorophore group and a fluorescence quenching leaving group selected from a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzenesulfonyl group, a nitro benzenesulfonyl group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group, and a gold particle conjugated to a sulfonyl group.

Livak teaches a quenched probe of an oligonucleotide with fluorescent dyes at opposite ends. In particular, the fluorescent dyes are a fluorescein reporter dye (e.g. 6-carboxyfluorescein (6-FAM) phosphoramidite) and a rhodamine quencher dye (e.g. 6-carboxytetramethylrhodamine succinimidyl ester (TAMRA NHS ester)). **Livak** does not teach a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzenesulfonyl group, a nitro benzenesulfonyl group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group, or a gold particle conjugated to a sulfonyl group as a fluorescence quenching leaving group, as claimed in the present application.

In view of the foregoing amendments and remarks, Applicant respectfully submits that the oligonucleotide probe taught by **Livak** is not the same as the fluorophore compound

of the present application. As such, the affirmed novelty rejection over Livak should be withdrawn.

New Claim Rejection – 35 USC §102(b)

Claim 12 is rejected under 35 USC §102(b) as allegedly being anticipated by Livak. Applicant respectfully traverses this rejection.

As discussed above, claim 1 has been amended to refer to a fluorescence quenching leaving group that is a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzenesulfonyl group, a nitro benzenesulfonyl group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group, or a gold particle conjugated to a sulfonyl group.

Claim 12, dependent upon claim 1, is directed to a composition comprising a fluorophore compound, which comprises a fluorophore group and a fluorescence quenching leaving group selected from a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzenesulfonyl group, a nitro benzenesulfonyl group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group and a gold particle conjugated to a sulfonyl group, as well as a nucleophilic group.

Livak, as discussed above, does not teach a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzenesulfonyl group, a nitro benzenesulfonyl group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group, or a gold particle conjugated to a sulfonyl group as a fluorescence quenching leaving group.

Although the purines and pyrimidines in **Livak**'s oligonucleotide probes might contain amine groups and amines are disclosed in the instant specification as being suitable nucleophiles, **Livak** does not teach or suggest the composition as recited in claim 12.

In view of the foregoing amendments and remarks, **Livak** does not anticipate claim 12. As such, the new ground of novelty rejection over **Livak** should be withdrawn.

New Claim Rejection – 35 USC §103(a)

Claim 9 is rejected under 35 USC §103(a) as allegedly being obvious in view of **Livak**. Applicant respectfully traverses this rejection.

As discussed above, claim 1 has been amended to refer to a fluorescence quenching leaving group that is a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzenesulfonyl group, a nitro benzenesulfonyl group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group, or a gold particle conjugated to a sulfonyl group.

Claim 9, dependent upon claim 1, is directed to a composition comprising a nucleic acid, which comprises a fluorophore group and a fluorescence quenching leaving group selected from a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzenesulfonyl group, a nitro benzenesulfonyl group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group, and a gold particle conjugated to a sulfonyl group, wherein the fluorescence quenching leaving group is attached to the 5' hydroxyl group of the nucleic acid.

Livak, as discussed above, does not teach a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzenesulfonyl group, a nitro benzenesulfonyl group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group, or a gold particle conjugated to a sulfonyl group as a fluorescence quenching leaving group. Although one of ordinary skill in the art might have recognized the usefulness of probe molecules having a quencher dye at the 5' hydroxyl position and a reporter dye at the 3' end based on **Livak**'s teaching that oligonucleotides with reporter and quencher dyes attached at opposite ends can be used as homogenous hybridization probes, he or she would not have arrived at the composition of instant claim 9.

In view of the foregoing amendments and remarks, **Livak** does not suggest the subject matter of claim 9. As such, the new ground of obviousness rejection over **Livak** should be withdrawn.

Allowable Subject Matter

The Examiner had previously objected to claim 15 as depending on rejected base claim 1. It would appear that the Examiner considers claim 15 allowable if rewritten to incorporate the limitations of the base claim. Applicant notes that claim 1 has been amended herein to incorporate the subject matter of claim 15. As such, claim 1, as presently amended, should be allowable.

In addition, the Examiner had previously allowed, in the second Advisory Action dated March 13, 2007, kit claims 40-49. Applicant respectfully requests that claims 40-49 remain allowed.

Rejoining of Non-Elected Groups and Species

Upon the allowance of product claims of Group I, *i.e.*, claims 1-14 as presently amended, Applicant respectfully requests that the method claims of Groups II and III, *i.e.*, claims 17-27 and 29-39, be rejoined for further examination. *See* MPEP §821.04. Applicant notes that claims 17, 21 and 25 have been amended herein to refer to the composition of claim 1. As amended, the withdrawn method claims incorporate all limitations of the product claims.

Also, Applicant respectfully requests that the claims directed to the non-elected species be rejoined with the claims directed to the elected species for further examination if the Examiner finds the claims directed to the elected species allowable upon considering the foregoing amendments and remarks. *See*, MPEP 809.02(a).

*****

This response is filed timely. The Commissioner is authorized to deduct the RCE fee of $405 from Howrey LLP Deposit Account No. 08-3038/12665.0024.NPUS01. Should any additional fees be required for any reasons relating to this document, the Commissioner is authorized to deduct such fees from the same deposit account.

Respectfully submitted,

J. Wendy Davis, Ph.D.
Reg. No. 46,393

Agent for Assignee
THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY

Customer No. 23369

HOWREY LLP
1111 Louisiana, 25th floor
Houston, TX 77002
(713) 787-1512 (Direct)

Date: August 11, 2008

EXPRESS MAIL MAILING LABEL

NUMBER: EV 958323446 US
DATE OF DEPOSIT: August 11, 2008

I hereby certify that this paper or fee is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria VA, 22313-1450.

Paula S. Linkhart

J|A|C|S
COMMUNICATIONS
Published on Web 02/13/2002

Quencher as Leaving Group: Efficient Detection of DNA-Joining Reactions

Shinsuke Sando and Eric T. Kool*
Department of Chemistry, Stanford University, Stanford, California 94305-5080

Received October 19, 2001

Recent experiments have described a number of strategies for detection of nucleic acids that involve a change in fluorescence intensity or emission wavelength.[1-3] Fluorescence-changing methods have the distinct advantage that unbound probe molecules can easily be distinguished from those bound to the desired target. Such approaches can be used either in solution or on solid supports, whereas static methods often cannot be used in solution, and typically require careful washing methods on solid supports. Approaches that rely on simple intensity variation by changes in quenching[3a,b] have the further advantage of freeing more spectral ranges so that multiple simultaneous probing can be achieved.

To date, the number of different quenching-based approaches to nucleic acid sensing is limited. Perhaps the most well-developed approach is that of "molecular beacons",[3c] which consist of hairpin-forming DNAs labeled in the stem with fluorophore and quencher. Binding to a complementary sequence results in opening of the hairpin and moving of the quencher away from the fluorophore. Beacons can be used in solution or in solid-supported approaches; however, their fluorescence change clearly depends on solution conditions (temperature, ionic strength) and so one must monitor conditions carefully. Moreover, methods that rely on hybridization alone are usually not as sequence selective as some recently developed DNA-sensing methods such as enzymatic approaches[4] or some nonenzymatic autoligation methods.[5,6] A few other intensity-changing strategies have also been described.[7]

Among the most sequence-selective DNA/RNA sensing strategies is the phosphorothioate/iodide DNA autoligation reaction.[5,8] This reaction is dependent on the presence of an exact complement to the two probes, and is more selective than T4 DNA ligase in many cases.[6] Here we show how, by replacement of iodide with a quencher acting as a leaving group, this approach can be adapted for fluorescence unquenching, resulting in a strong signal change when two DNA strands are joined.

The strategy of the quenched autoligation (QUAL) probes is simple. A dabsyl group activates the 5′ hydroxyl as a leaving group when a phosphorothioate nucleophile (from another DNA strand) attacks at the ultimate 5′ carbon. This reaction is expected to be promoted by the presence of a complementary DNA strand, which brings the two reactive groups into close proximity. To test this approach we selected thymidine to modify with the quencher/leaving group, which is conveniently available commercially as the sulfonyl chloride derivative. To ensure relatively efficient quenching we placed a standard fluorophore (a commercial fluorescein C-5-alkenyl conjugate of dU) nearby, at a distance of three nucleotides from the 5′ terminus in the quenched electrophilic probes. These 13mer probes were prepared using standard phenoxyacetyl base protection chemistry to avoid partial loss of the dabsyl group during deprotection. They were purified by HPLC and characterized by MALDI-TOF mass spectrometry. These were reacted with 7mer

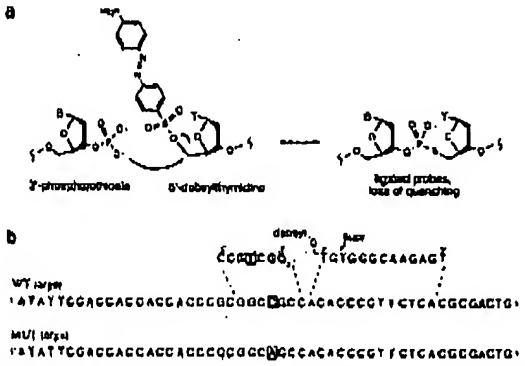

Figure 1. (a) Structure of the dabsyl-thymidine nucleoside and mechanism of dabsyl displacement. (b) Schematic showing how autoligation of two DNA strands on a complementary DNA template (the analyte) leads to displacement of "dabsylate" and loss of quenching. Sequences are as shown, and are taken from the H-*ras* protooncogene.

probes carrying a phosphorothioate group at the 3′ terminus.[9] To test for sequence selectivity in a biologically relevant context, we chose probes complementary to adjacent sites in the H-*ras* gene, such that the known C → A transversion[10] in codon 12 falls at the center of the 7mer binding site. The 7mer MUT probes were fully complementary to the C → A mutant (MUT) sequence. Target DNAs were 50nt in length and corresponded to the sequence of the H-*ras* gene sense strand.

Reactions were initially tested in solution, following the ligation by gel electrophoresis. Results showed clearly that the autoligation reaction proceeded smoothly and with high conversion. Figure 2a shows a stained PAGE gel image, revealing ligated product when the target sequence is fully complementary, and no discernible product when it is different by one nucleotide. Conversion after 7 h is estimated at 80% in the fully complementary case. Significantly, a fluorescence image of the same gel shows (Figure 2b) that while the starting materials are only very weakly fluorescent (due to incomplete quenching by dabsyl), the product band shows robust emission, consistent with a substantial loss of quenching. A faint ligated band is also visible just beneath the major product; this we attribute to ligation by an (N − 1)mer contaminant from DNA synthesis.

We then monitored the same reaction in real time by following fluorescence emission at 520 nm (excitation 495 nm) using the 7mer MUT probe (Figure 2c). Monitoring the change in fluorescein emission showed a large increase in intensity (ca. 100-fold, implying a 99.0% quenching efficiency at 520 nm for the starting probe). Varying the temperature from 15 to 37 °C revealed that the rate reached a maximum near 25 °C, which corresponds to the approximate melting temperature of the probes bound to the target (data not shown). The sequence selectivity of the reaction was

* To whom correspondence should be addressed. E-mail: kool@stanford.edu.

10.1021/ja017338o CCC: $22.00

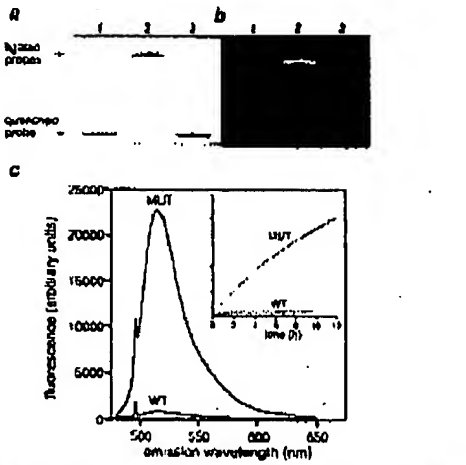

Figure 2. Analysis of dabsyl-mediated autoligations by gel electrophoresis and fluorescence. (a) Stained gel showing starting materials and ligated product: lane 1, no target; lanes 2 and 3, with MUT and WT target, respectively. Conditions: 1.3 μM dabsyl-labeled 13mer probe, 3.9 μM 7mer MUT probe and target DNA concentrations in a pH 7.0 Tris-borate buffer containing 10 mM $MgCl_2$ at 37 °C; (b) fluorescence image of the same gel, showing weak fluorescence of quenched 13mer probe and strong fluorescence of ligated product band; (c) emission spectra of reactions with MUT and WT DNA after 24 h (inset: timecourse monitored at 520 nm). Conditions: 100 nM dabsyl-labeled 13mer probe, 300 nM 7mer MUT probe and target DNA concentrations in a pH 7.0 Tris-borate buffer containing 10 mM $MgCl_2$ at 25 °C.

approximately 35-fold based on relative peak areas with WT and MUT target DNAs, resulting in a T−A pair or a T−C mismatch at the mutation site in codon 12. The overall rate of the reaction was similar to that of previously described iodide-mediated reactions.[6a,8] The 5′-dabsyl group, like 5′-iodide, has the advantage of limited reactivity (relative to tosylate[6b,c]) under DNA deprotection conditions.

Since many recent genetic analysis methods rely on probes affixed to beads, slides, and other surfaces, we then explored whether this reaction could take place on a solid support. This was tested by synthesizing a 7mer MUT probe on 90 μm diameter polystyrene beads (1000 Å pore size) using commercially available reverse (5′ — 3′) phosphoramidites, and placing a 3′ phosphorothioate moiety on the final 3′ hydroxyl group. A hexaethylene glycol linker was used to alleviate potential crowding problems near the polymer surface. Such beads then have the potential to autoligate a 13mer electrophile probe to themselves, in the presence of the correct target DNA. This is expected to result in the beads becoming fluorescent, as the dabsylate group is lost into solution and the nearby fluorescein label loses quenching.

The solid-phase autoligations were monitored by imaging under a fluorescence microscope. Results showed that the reaction proceeds on the polystyrene beads much as it does in solution (Figure 3). At the start of the reaction the beads are dark and the solution shows faint green fluorescence due to a small amount of emission from the quenched 13mer probe. As the reaction proceeds the beads become progressively brighter, reaching half-maximum after ca. 20 h.

These early experiments allow us to conclude that the use of a "dabsylate" leaving group on probes enables facile detection of DNA strand-joining reactions. The reaction retains the high sequence selectivity of the autoligation approach, and proceeds well in solution or on a solid support. Like molecular beacons, this quencher/leaving group approach results in an increase in intensity

Figure 3. Dabsyl-mediated autoligation of DNAs on solid support. Photographs show beads after 24 h of incubation with (a) MUT or (b) WT target using a dabsyl/fluorescein 13mer probe at 25 °C. No washing was done. Conditions are given in the Supporting Information.

on detection of a complementary sequence, and like that method, one can in principle adapt it to multicolor detection for simultaneous (multiplex) sensing of more than one sequence.[12] It should be noted, however, that this autoligation reaction is slower than simple hybridization-based approaches, and so is not suitable for a rapid-timescale application such as real-time PCR reporting. However, the present strategy offers the significant advantage over hybridization that the signal, once generated, is permanent and does not depend on temperature or buffer conditions. Moreover, DNA autoligation reactions are more sensitive to single-nucleotide differences than are most, if not all, hybridization-based approaches.

Future work will be directed at applying this unquenching autoligation strategy to simultaneous multiple-sequence sensing, and to cellular imaging of DNAs and RNAs.

Acknowledgment. This work was supported by the U.S. National Institutes of Health (GM62658) and by a grant from the U.S. Army Research Office. S.S. acknowledges a postdoctoral fellowship from the JSPS.

Supporting Information Available: Details of synthesis and methods (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Cardullo, R. A.; Agrawal, S.; Flores, C.; Zamecnik, P. C.; Wolf, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8790. (b) Heid, C. A.; Stevens, J.; Livak, K. J.; Williams, P. M. *Genome Res.* **1996**, *6*, 986. (c) Paris, P. L.; Langenhan, J.; Kool, E. T. *Nucleic Acids Res.* **1998**, *26*, 3789. (d) Masuko, M.; Ohtani, H.; Ebata, K.; Shimadzu, A. *Nucleic Acids Res.* **1998**, *26*, 5408. (e) Masuko, M.; Ohuchi, S.; Sode, K.; Ohtani, H.; Shimadzu, A. *Nucleic Acids Res.* **2000**, *28*, e34.

(2) Samiotaki, M.; Kwiatkowski, M.; Parik, J.; Londegren, U. *Genomics* **1994**, *20*, 238.

(3) (a) Tyagi S.; Kramer F. R. *Nature Biotechnol.* **1996**, *14*, 303. (b) Dubertret, D.; Calame, M.; Libchaber, A. *Nature Biotechnol.* **2001**, *19*, 365. (c) Zhang, P.; Beck, T.; Tan, W. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 402.

(4) (a) Wu, D. Y.; Wallace, R. B. *Gene* **1989**, *76*, 245. (b) Luo, J.; Bergstrom, D. E.; Barany, F. *Nucleic Acids Res.* **1996**, *24*, 3071. (c) Pastinen, T.; Kurg, A.; Metspalu, A.; Peltonen, L.; Syvanen, A. C. *Hum. Mutat.* **1996**, *7*, 346. (d) Dubiley, S.; Kirillov, E.; Mirzabekov, A. *Genome Res.* **1997**, *7*, 606. (e) Shumaker, J. M.; Tollot, J. J.; Filbin, K. J.; Montague-Smith, M. P.; Pirrung, M. C. *Clin. Chem. Lab. Med.* **2000**, *38*, 165. (f) Stevens, P. W.; Hall, J. G.; Lyamichev, V.; Neri, B. P.; Lu, M. C.; Wang, L. M.; Smith, L. M.; Kelso, D. M. *Nucleic Acids Res.* **2001**, *29*, U13.

(5) (a) Gryaznov, S. M.; Letsinger, R. L. *J. Am. Chem. Soc.* **1993**, *115*, 3808. (b) Gryaznov, S. M.; Schultz, R.; Chaturvedi, S. K.; Letsinger, R. L. *Nucleic Acids Res.* **1994**, *22*, 2366.

(6) (a) Xu, Y.; Kool, E. T. *Nucleic Acids Res.* **1999**, *27*, 875. (b) Xu Y.; Karalkar, N. B.; Kool, E. T. *Nature Biotechnol.* **2001**, *19*, 148.

(7) (a) Friedman, A. E.; Chambron, J.-C.; Sauvage, J.-P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960. (b) Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518.

(8) (a) Xu, Y.; Kool, E. T. *Nucleic Acids Res.* **1998**, *26*, 3159. (b) Xu, Y.; Kool, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 9040.

(9) (a) Cook, A. F. *J. Am. Chem. Soc.* **1970**, *92*, 190. (b) Chladek, S.; Nagyvary, J. *J. Am. Chem. Soc.* **1972**, *94*, 2079.

(10) Reddy, E. P.; Reynolds, R. K.; Santo, E.; Barbacid, M. *Nature* **1982**, *300*, 149.

(11) Herrlein, M. K.; Nelson, J. S.; Letsinger, R. L. *J. Am. Chem. Soc* **1995**, *117*, 10151.

(12) Tyagi, S.; Bratu, D. P.; Kramer, F. R. *Nature Biotechnol.* **1998**, *16*, 49.

JA017328S